蝶と蛾 *Trans. lepid. Soc. Japan* **55** (4): 275–279, September 2004

Photoperiod effects on pupal development in two tropical *Papilio* butterflies (Lepidoptera, Papilionidae)*

Masanobu YOSHIO** and Minoru ISHII

Entomological Laboratory, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai, Osaka, 599-8531 Japan

Abstract Larvae of Sabahan *Papilio memnon* L. and *P. polytes* L. were reared under a photoperiod of 12L-12D or 14L-10D at 20°C. In both species, photoperiods affected pupal development, although there was no significant difference in the mean larval stages between photoperiodic conditions. In *P. memnon*, the pupal stage under the short day (23–27 days) was slightly but significantly longer than that under the long day (23–24 days). In *P. polytes*, four of nine individuals prolonged their pupal stages under the short day (29–92 days), while all individuals developed without arrest in development under the long day (19–21 days). The results show that these *Papilio* butterflies inhabiting the tropics near the equator have the ability to delay pupal development in response to a short photoperiod at the moderate temperature.

Key words Development, pupal diapause, photoperiod, *Papilio memnon*, *Papilio polytes*, tropical region, Sabah.

Introduction

In temperate insects, diapause is regarded as one of the important adaptations for surviving adverse seasons, and is mainly controlled by photoperiod and temperature (Danilevsky, 1965; Danks, 1987). In *Papilio* butterflies, it has been reported in several temperate species that pupal diapause is induced by larval photoperiod (Oliver, 1969; Hidaka & Hirai, 1970; Ichinosé, 1974; Ichinosé & Negishi, 1979; Ishii, 1977; James, 1988; Yoshio & Ishii, 1998).

In tropical regions, on the other hand, insects are subjected to nearly constant conditions of temperature enabling them to develop throughout the year. However, it is known that tropical insects including butterflies show such seasonalities as population fluctuation and production of seasonal morphs (Owen, 1971; Young, 1982; Wolda 1982; Ishii, 1994; Braby, 1995). Denlinger (1986) reviewed dormancy in tropical insects and concluded that diapause is not a rare event in tropical regions.

Evidence for diapause has been also reported in tropical papilionids (Singh, 1993). However, only a few studies have been made so far on diapause in tropical *Papilio* species, so that the factor or factors for its regulation are still unclear. Ishii (1987) reported that some individuals of *P. demoleus* L. from Sabah, Malaysia, entered pupal diapause under a short photoperiod of 10L-14D at 20°C, and suggested that moderate rearing temperature might induce diapause. We carried out an experiment to elucidate the effect of photoperiod on larval and pupal development in two tropical papilionids, *P. memnon* L. and *P. polytes* L.

---

*Permission to import materials of foreign butterflies was given by the Kobe Plant Protection Station of the Ministry of Agriculture, Forestry and Fisheries of Japan (No. 7K106).

**Present address: Division of Radiobiology and Environmental Science, Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka, 599-8570 Japan.

Materials and methods

Three middle instar larvae of *P. memnon* and four of *P. polytes* were collected at Ranau (5.5°N) in Sabah, Malaysia in late February, 1995 (Fig. 1). These larvae were reared individually in 200-m*l* clear plastic cups on fresh leaves of citrus, *Citrus* spp., under semi-natural conditions during our period in Sabah, and under a long photoperiod of 14L-10D at 25±1.5°C in the laboratory of Osaka Prefecture University, Sakai, Japan. As for *P. memnon*, the final instar larvae were reared in 500-m*l* cups until adult emergence. All the larvae developed into adults without any arrest in development in these conditions as reported by Ishii (1987). Eggs were obtained from females of both species mated by using the hand-pairing method (Clarke & Sheppard, 1956).

Twenty three and 28 eggs were obtained from the females of *P. memnon* and *P. polytes*, respectively. Newly hatched larvae were reared according to Yoshio & Ishii (1996) on an artificial diet at 20±1.5°C. Since Sabah is located at about 5°N and the daylength including civil lights at a latitude of 5°N ranges from 12 hr 36 min to 13 hr 11 min (Beck, 1980), we adopted two photoperiods of 14L-10D and 12L-12D as being slightly longer and shorter than the local natural daylengh. Pupae thus obtained were kept under the same conditions as the larval stage until adult emergence. Larval and pupal stages were recorded individually.

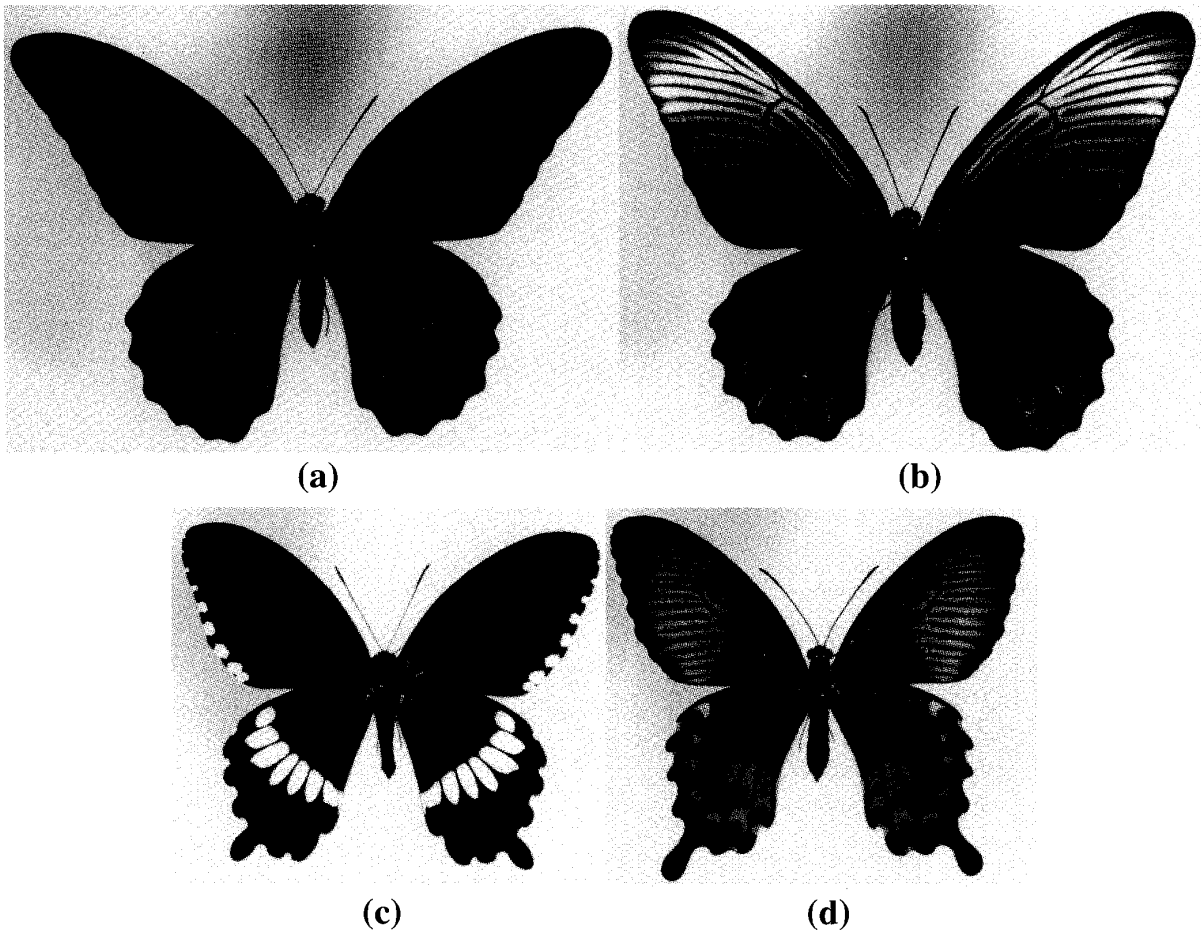

Fig. 1. Male and female adults of *Papilio memnon* L. (a, b), and *P. polytes* L. (c, d) from Sabah, Malaysia.

Table 1. Mean larval stages (mean±SD days) of two Sabahan *Papilio* species, *P. memnon* and *P. polytes*, under long day (14L-10D) and short day (12L-12D) at 20°C.

	14L-10D	12L-12D	*P**
P. memnon	32.5±1.9 (*n*=9)	32.7±2.7 (*n*=10)	*P*>0.05
P. polytes	32.9±2.0 (*n*=13)	32.2±1.5 (*n*=11)	*P*>0.05

*Significance between means under two photoperiods by t-test.

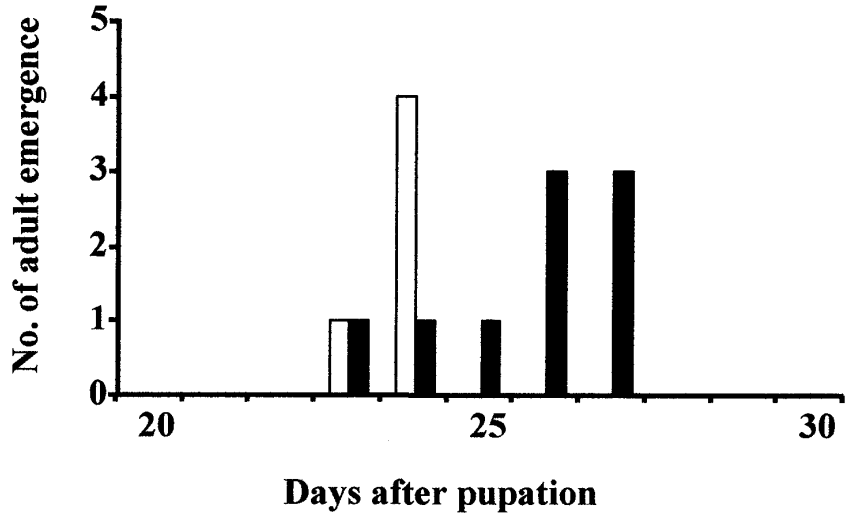

Fig. 2. Frequency distribution of the pupal stages of the Sabahan *P. memnon* reared under 14L-10D (open columns) and 12L-12D (closed ones) at 20°C.

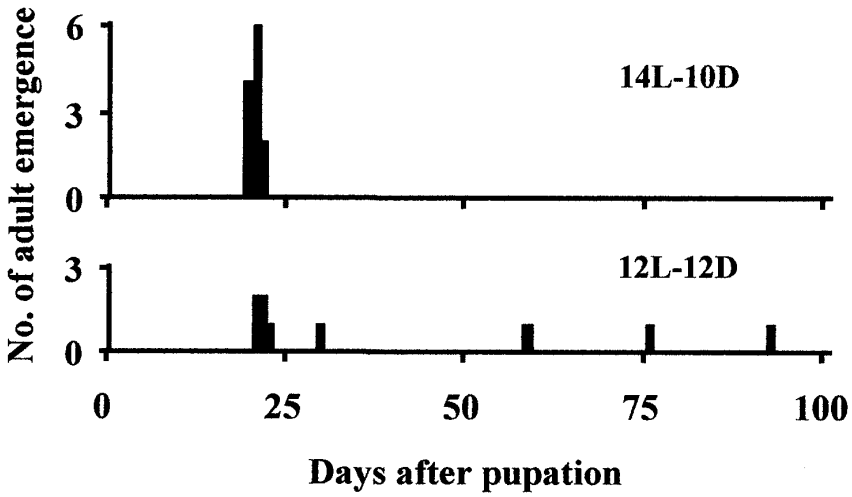

Fig. 3. Frequency distribution of the pupal stages of the Sabahan *P. polytes* reared under 14L-10D (upper) and 12L-12D (lower) at 20°C.

Results and discussion

There was no significant difference in the mean larval stages between photoperiods in either *P. memnon* or *P. polytes* (*P*>0.05, Table 1): The mean larval stages under 14L-10D and

12L-12D at 20°C were 32.5 and 32.7 days in *P. memnon*, 32.9 and 32.2 days in *P. polytes*, respectively. Ishii (1987) reported that photoperiod did not influence larval development in three Sabahan papilionids, *P. demoleus*, *P. memnon* and *P. polytes*, at a temperature of 25°C. Our results showed that larval development in *P. memnon* and *P. polytes* was not affected by photoperiod even at a temperature of as low as 20°C.

However, there was a marked difference in the pupal stage of both species between the two photoperiodic conditions. Figure 2 shows the frequency distribution of pupal stages of *P. memnon* reared under 14L-10D and 12L-12D at 20°C. The pupal stage under 12L-12D (23–27 days, N=9) was slightly but significantly longer than that under 14L-10D (23–24 days, N=5) (Mann-Whitney U-test, P<0.05).

In *P. polytes*, although all the individuals emerged within 21 days (19–21 days, N=12) after pupation under 14L-10D, the pupal stages under 12L-12D varied between 20 and 92 days (Fig. 3): Four of nine individuals which pupated showed an extension of pupal stage, and emerged as adults 29, 58, 75 and 92 days after pupation, respectively.

The results show that Sabahan *P. memnon* and *P. polytes* have the ability to respond to a short photoperiod as 12L-12D by extending the pupal stage if combined with a moderate temperature as 20°C. Especially in *P. polytes*, there was significant difference in the ratio of individuals that extended the pupal stage between photoperiods (Fisher's exact probability test, P<0.05), and such an extension of pupal stage could be explained only by the induction of diapause.

It is intriguing that the Sabahan *Papilio* species showed a photoperiodic response, although Sabah is located at a low latitude of around 5°N where the seasonal variation of daylength is small. Denlinger (1986) suggested that the change in photoperiod may not serve as a seasonal cue for inducing diapause in insects inhabiting tropical regions near the equator. Our results, however, clearly show that photoperiod would act as an important seasonal cue of diapause regulation even quite near the equator at least in a Sabahan butterfly, *Papilio polytes*, under moderate temperature.

Acknowledgment

We are grateful to Prof. T. Yagi, Dr T. Hirowatari and Mr N. Hirai of Osaka Prefecture University for their invaluable comments and assistance. Thanks are also due to Dr H. Tanaka of the Agricultural, Food and Environmental Sciences Research Center of Osaka Prefecture, and Dr R. Mochioka of Kagawa University for supplying citrus leaves.

References

Beck, S. D., 1980. *Insect Photoperiodism* (2nd Edn). Academic Press, New York.

Braby, M. F., 1995. Reproductive seasonality in tropical satyrine butterflies: strategies for the dry season. *Ecol. Ent.* **20**: 5–17.

Clarke, C. A. & P. M. Sheppard, 1956. Hand-pairing of butterflies. *Lepid. News* **10**: 47–53.

Danilevsky, A.S., 1965. *Photoperiodism and seasonal Development of Insects* (English Edn). Oliver & Boyd, Edinburgh and London (Translated from Russian by J. Johnston assisted by N. Waloff).

Danks, H. V., 1987. *Insect Dormancy: an ecological Perspective*. Biological Survey of Canada, Ottawa.

Denlinger, D.L., 1986. Dormancy in tropical insects. *A. Rev. Ent.* **31**: 239–264.

Hidaka, T. & Y. Hirai, 1970. Effect of non-24-hour photoperiod and light interruption of the dark phase on diapause determination in *Papilio xuthus* L. *Proc. Japan Acad.* **46**: 541–545.

Ichinosé, T., 1974. Pupal diapause in some Japanese papilionid butterflies, with special reference to the difference in photoperiodic response between the diapausing pupae of *Papilio maackii tutanus* Fenton and *P. xuthus* Linnaeus. *Kontyû* **42**: 439–450.

Ichinosé, T. & H. Negishi, 1979. Pupal diapause in some Japanese papilionid butterflies II. The difference in the induction of diapause between the two subspecies of *Papilio protenor* Cramer. *Ibid.* **47**: 89–98.

Ishii, M., 1977. Photoperiodic reaction of the Hokkaido population of *Papilio bianor dehaanii* C. et R. Felder (Lepidoptera: Papilionidae). *Tyô Ga* **28**: 115–116.

————, 1987. Diapause potential in tropical papilionids (Lepidoptera: Papilionidae). *Appl. Ent. Zool.* **22**: 114–115.

————, 1994. Factors determining larval, pupal and adult polyphenisms in the tropical butterfly, *Catopsilia pomona* (F.) (Lepidoptera, Pieridae). *Tyô Ga* **45**: 105–112.

James, D. G., 1988. Induction of pupal diapause in *Papilio aegeus aegeus* Donovan and *Graphium sarpedon choredon* (C. & R. Felder) (Lepidoptera: Papilionidae). *Aust. Ent. Mag.* **15**: 39–44.

Oliver, C. G., 1969. Experiments on the diapause dynamics of *Papilio polyxenes*. *J. Insect Physiol.* **15**: 1579–1589.

Owen, D. F., 1971. *Tropical Butterflies*. Clarendon Press, Oxford.

Singh, S. P., 1993. Species composition and diapause in citrus butterflies. *J. Insect Sci.* **6**: 48–52.

Wolda, H., 1982. Seasonality of Homoptera on Barro Colorado Island. *In* Leigh, E. G. Jr, Rand, A. S. and D. M. Windsor (Eds), *The Ecology of a tropical Forest. Seasonal Rhythms and long-term Changes*: 319–330. Smithsonian Institution Press, Washington.

Young, A. M., 1982. *Population Biology of tropical Insects*. Plenum Press, New York and London.

Yoshio, M. & M. Ishii, 1996. Rearing larvae of the great mormon butterfly, *Papilio memnon* L. (Lepidoptera: Papilionidae), on artificial diet. *Jap. J. Ent.* **64**: 30–34.

————, 1998. Geographical variation of pupal diapause in the great mormon butterfly, *Papilio memnon* L. (Lepidoptera: Papilionidae), in western Japan. *Appl. Ent. Zool.* **33**: 281–288.

摘　　要

熱帯産アゲハチョウ類2種の蛹発育に及ぼす光周期の影響 (吉尾政信・石井　実)

温帯および亜熱帯産の*Papilio*属アゲハチョウ類では，幼虫期の光周期によって蛹休眠が誘導されることが知られている．一方，熱帯産のアゲハチョウ類の休眠性に関する研究は少なく，休眠の誘導条件などについては不明な点が多い．Ishii (1987) は，オナシアゲハ*Papilio demoleus* L. のサバ個体群 (マレーシア，サバ州) の幼虫を20°C, 10時間日長で飼育することにより，蛹休眠を誘導しているが，長日区を設けていないため日長の役割については明確ではない．そこで本研究では，熱帯産アゲハチョウ類の休眠性における光周期の役割を明らかにするために実験を行った．

1995年2月にサバ州でナガサキアゲハ*P. memnon* L. とシロオビアゲハ*P. polytes* L. の幼虫を採集し，柑橘類*Citrus* spp. の生葉で飼育した．羽化した成虫をハンドペアリング法によって交尾させた後に採卵し，孵化した幼虫を20°Cの12時間および14時間日長で飼育し，幼虫および蛹期間を記録した．

その結果，両種とも幼虫期間は約1ヶ月で日長による差はなかったが，蛹期間については日長条件で差が認められた．ナガサキアゲハの蛹期間は，14時間日長で23–24日，12時間日長では23–27日で，わずかではあるが短日で有意に長かった．シロオビアゲハについては，14時間日長では19–21日であったが，12時間日長では蛹化後20–22日に羽化したグループと，羽化までに29–92日を要したグループに分かれた．すなわち，シロオビアゲハでは20°Cの短日条件下では休眠する個体が存在した．シロオビアゲハのサバ個体群は25°Cでは短日条件下 (10時間日長) でも休眠に入らなかったが (Ishii, 1987), 20°Cという熱帯では冷涼な気温と短日の組み合わせによって蛹休眠が誘導されることが明らかになった．Denlinger (1986) は，1年を通じて日長の変化の小さい赤道付近では，光周期は休眠誘導の季節信号として機能しないことを示唆しているが，少なくともシロオビアゲハのサバ個体群においては光周期は重要な季節信号であることが示された．

(Accepted May 13, 2004)

Published by the Lepidopterological Society of Japan,
5-20, Motoyokoyama 2, Hachioji, Tokyo, 192-0063 Japan